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The *UPF1* RNA Surveillance Gene is Commonly Mutated in Pancreatic Adenosquamous Carcinoma

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Abstract

Pancreatic adenosquamous carcinoma (ASC) is an enigmatic and aggressive tumor that has a worse prognosis and higher metastatic potential than its adenocarcinoma counterpart. Here we report that ASC tumors frequently harbor somatically acquired mutations in the *UPF1* gene, which encodes the core component of the nonsense-mediated RNA decay (NMD) pathway. These tumor-specific mutations alter *UPF1* RNA splicing and perturb NMD, leading to upregulated

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AUTHOR CONTRIBUTIONS

C.L., R.K., Y.Z., F.S., C.W., M.S., Y.W., G.X., L.L., J.Z., and Y.M. performed the experiments. Y.Z., Y.J., G.L., W.F., M.Z., M.A.V. and M.J. provided clinical samples and expertise on pancreatic cancer. R.K. and C.L. prepared the figures and tables and wrote the manuscript. Y.L. and M.F.W. equally contributed to this study by designing and supervised all experiments and assisting in writing the manuscript.

SUPPLEMENTARY INFORMATION

Includes Supplementary Figures 1 to 5, Supplementary Tables 1 to 7, Methods, and Supplementary References.

COMPETING FINANACIAL INTERESTS

The authors declare no competing financial interests.

levels of NMD substrate mRNAs. *UPF1* mutations are the first known unique molecular signatures of ASC.

There has been little progress in understanding pancreatic ASC since these aggressive tumors were first described over a century ago[1,2]. One problem is that no mutations unique to this class of pancreatic tumors have been identified. Thus, while oncogenes and tumor suppressor genes have been shown to be altered in ASC—including *K-RAS* and *p53*—these same genes are also abnormal in other forms of pancreatic cancer[3–5]. In this communication, we report that ASC pancreatic tumors have somatic mutations in *Up-frameshift 1* (*UPF1*), which encodes an RNA helicase essential for a highly conserved RNA degradation pathway called nonsense-mediated RNA decay (NMD)[6]. We were initially led to investigate the possibility that NMD had a role in ASC because reverse transcription-polymerase chain reaction (RT-PCR) analysis showed that an ASC tumor (TU) with wild-type *p53* alleles expressed an abnormally large band that was not detectable in the adjacent normal pancreas (NP) (Supplementary Fig. 1a). Sequence analysis of this large band demonstrated that it was generated by alternatively splicing using a non-canonical splice donor in intron 6 and a non-canonical splice acceptor in intron 10, which both share 14 nt of sequence identity with the splice donor (Supplementary Fig. 1b). Quantitative PCR (qPCR) analysis demonstrated that the alternatively spliced p53 mRNA was only detectably expressed in tumor tissue, not adjacent normal tissue, while the normally spliced p53 mRNA was expressed at similar levels in the tumor and adjacent normal tissue (Supplementary Fig. 1c).

The alternative splicing event creating this p53 isoform generates an in-frame premature termination codon (PTC) in intron 6 (Supplementary Fig. 1b). Because NMD degrades mRNAs with PTCs[6,7], the selective accumulation of this “alt-PTC-IVS6-p53” mRNA in the tumor tissue but not the adjacent normal tissue, raised the possibility that the tumor tissue had deficient NMD. To test this, we screened ASC tumor samples for mutations in core NMD genes. We found genomic mutations in the *UPF1* gene in ASC tumors from 18 of 23 patients (see TU in Fig. 1a), whereas the 3 other NMD genes we tested—*UPF2*, *UPF3A*, and *UPF3B*—did not have detectable mutations (Supplementary Table 1). The *UPF1* mutations were somatic in origin, since they were not present in matched normal pancreatic tissues from these 18 patients (see NP in Fig. 1a). *UPF1* mutations were also not present in 50 non-ASC pancreatic and SCC lung tumors that we tested (Supplementary Table 2). Together, these results suggest that *UPF1* mutations are a unique signature of ASC pancreatic tumors.

The point mutations in the ASC tumors clustered in two regions of the *UPF1* gene (Fig. 1b). Surprisingly, these mutations were nearly equally distributed in the exons and introns in these two regions. This raised the possibility that they alter *UPF1* splicing by disrupting intronic splicing enhancers (ISEs) and exonic splicing enhancers (ESEs), which are essential for the inclusion of a subset of exons during splicing[8,9]. In support of this, a subset of the mutations in the ASC tumors disrupted predicted ESEs/ISEs (Fig. 1b and Supplementary Fig. 2). To directly test whether the ASC mutations disrupt *UPF1* splicing, we generated *UPF1* mini-gene constructs corresponding to the two regions mutated in ASC tumors (Fig. 2a,b). We then introduced, by site-specific mutagenesis, the mutations found in 15 of the

patients. Transfection analysis showed that the wild-type versions of the two mini-gene constructs expressed normally spliced mRNA that included all exons, as determined by direct sequencing of the bands generated by RT-PCR (Fig. 2c,d). No other bands were detected, indicating that most of the mature mRNA generated by these mini-gene constructs was normally spliced. In contrast, all 15 mutant mini-genes expressed alternatively spliced transcripts (Fig. 2,d). While none of the patient's mutations completely eliminated normal splicing, the ratio of alternatively spliced-to-normally spliced mRNA was 10 to 1 or higher in many cases. As confirmation, analysis of endogenous *UPF1* splicing in 2 of 2 frozen tumor samples revealed an alternatively spliced *UPF1* mRNA that was not present in the adjacent normal tissue (Fig. 2e). We conclude that ASC-specific mutations in the *UPF1* gene trigger alternative splicing of *UPF1* pre-mRNA.

The *UPF1* alternative splicing events caused by mutations in the exon 10/11 region eliminate a portion of UPF1's RNA helicase domain essential for UPF1 function[6], while mutations in the exon 21/23 region truncate the carboxy-terminal region of UPF1, which contains [S/T]Q motifs phosphorylated by SMG1, a serine/threonine kinase required for NMD[10] (Figs. 1b and 2c). Given that essential UPF1 domains are lost, these mutant forms of UPF1 are likely to have lost their function or have dominant-negative activity. In the case of the latter, inhibited NMD would be predicted to occur when only one *UPF1* allele is mutated. In support of this possibility, mutations in various regions of UPF1 have previously been shown to confer dominant-negative activity[11–13]. With regard to the former, we found that at least one ASC tumor (from patient 22) had mutations in both *UPF1* alleles. Several other ASC tumors have multiple *UPF1* mutations (Supplementary Table 1), consistent with some of them also having mutations in both *UPF1* alleles. In further support of this notion, we observed little or no UPF1 expression in many ASC tumors compared to adjacent normal tissue, as demonstrated by immunohistochemical (IHC) analysis (Fig. 2f and Supplementary Table 1). As direct evidence that NMD is inhibited in ASC tumors, we found that the NMD substrates, *ATF3* and *MAP3K14* mRNA[14], were dramatically upregulated in tumor tissue relative to adjacent normal tissue (Supplementary Fig. 3).

Since perturbation of NMD is known to dysregulate ~3 to 10% of mRNAs in a variety of cell lines and organisms[6,14,15], it is likely this will also be the case of ASC tumors, which could shift the balance towards a more malignant phenotype[16,17]. One candidate NMD substrate that could serve in this capacity is the alternatively spliced p53 transcript—*alt-PTC-IVS6-p53*—that we identified above (Supplementary Fig. 1), as we found it encoded a protein with dominant-negative activity (Supplementary Fig. 4).

The discovery of mutations in the *UPF1* gene in ASC tumors represents the first known example of genetic alterations in a NMD gene in human tumors. To our knowledge, *UPF1* is also the first gene known to be selectively mutated in pancreatic ASC tumors. Other genes mutated in these tumors, including *p53* and *K-RAS* (see Supplementary Fig. 5 and Supplementary Table 1) are also mutated in other pancreatic tumor types[4,5]. We note that it is possible that *UPF1* mutations in ASC tumors have broad disruptive effects that that extend beyond NMD, including Staufen-mediated RNA decay[18]. Our finding that *UPF1* is selectively mutated in pancreatic ASC suggests that this feature will be useful for diagnosis

of this type of tumor and also raises the possibility that ASC patients can benefit from therapy designed to target NMD substrates[19,20].

METHODS

Methods are available in the Supplementary Information section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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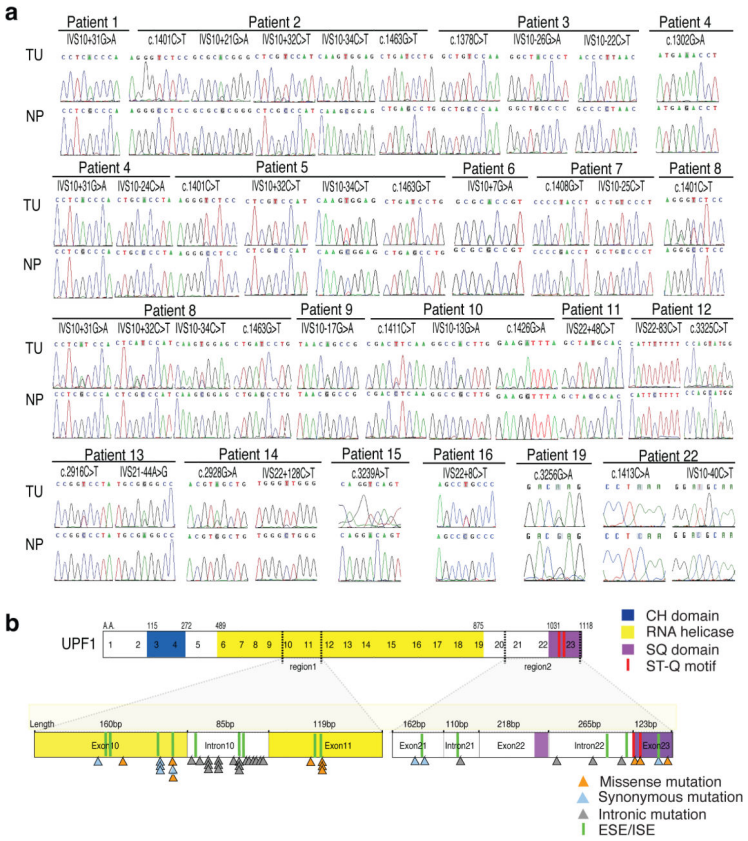

Figure 1.

Somatic *UPF1* mutations in ASC tumors. (**a**) Chromatograms of *UPF1* DNA sequences from tumor (TU) and normal adjacent pancreatic tissue (NP). A total of 36 single-base substitutions in genomic *UPF1* DNA were found in the tumors; each patient had between one to five point mutations. All NP samples had wild-type *UPF1* sequences, indicating mutations were somatic in origin. (**b**) Top: schematic of UPF1 protein domains. Bottom: the type and location of mutations found in the two regions of *UPF1* that harbored mutations. ESEs and ISEs are exonic and intronic splicing enhancers, respectively (sequences are provided on Supplementary Figure 2).

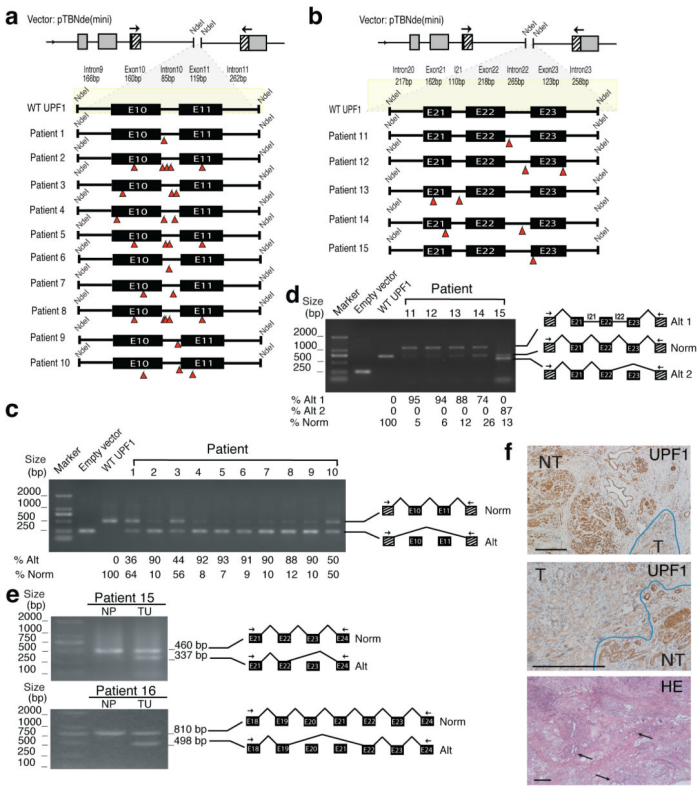

Figure 2.

ASC-specific *UPF1* mutations trigger alternative *UPF1* RNA splicing. (**a**) The indicated region of human *UPF1* (nt 22,779-23,570, RefSeq accession number NC_000019.9) was cloned into the *Nde*I site of the *pTBNde* mini-gene construct. All mutations were generated by site-directed mutagenesis to match those in the indicated patient's tumors. (**b**) The indicated region of human *UPF1* (nt 33,138-34,490) was cloned and mutated as described in panel **a**. (**c**) RT-PCR analysis of HEK293 cells transfected with the constructs shown in panel **a** (primer locations are indicated by the arrows). Direct sequencing of the large (792 nt) and small (239 nt) bands indicated that they correspond to normally spliced and exon-skipped transcripts, respectively. The numbers below the gel are the average values from five independent transfections. (**d**) RT-PCR analysis performed as in panel **c**. Direct sequencing of the bands in the gel indicated they were derived from mRNA spliced in the manner shown in the schematic. The bands corresponding to normally spliced, alt 1, and alt 2 mRNA had lengths of 742, 1117, and 619 nt, respectively. The numbers below the gel are the average values from five independent transfections. (**e**) RT-PCR analysis of normal pancreas (NP) and ASC frozen samples (TU) from patients 15 and 16. RT-PCR sequencing results are indicated as schematics next to the gels. (**f**) Immunohistochemical analysis of UPF1 staining in ASC tumor samples from patient 15. Tumor (T) and normal tissue (NT) are indicated. Bottom panel shows H & E staining, with arrows pointing to the adenocarinoma component in this ASC tumor. The scale bar represents 200 micrometers on each picture.